FINAL REPORT

Title: Managing with fire to promote the recently listed Florida Bonneted Bat, *Eumops floridanus*

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PI: Holly K. Ober
Affiliation: University of Florida

Co-PI: Robert A. McCleery
Affiliation: University of Florida

Post-doctoral Researcher: Elizabeth C. Braun de Torrez
Affiliation: University of Florida

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List of Abbreviations/Acronyms

BICY = Big Cypress National Preserve

BWWMA = Babcock-Webb Wildlife Management Area

EUFL = *Eumops floridanus* = Florida bonneted bat

FPNWR = Florida Panther National Wildlife Refuge

FSPSP = Fakahatchee Strand Preserve State Park

FWC = Florida Fish and Wildlife Conservation Commission

USFWS = United States Fish and Wildlife Service

Keywords: acoustic surveys, bat conservation, endangered species, *Eumops floridanus*, fire return interval, fire seasonality, Florida bonneted bat, foraging ecology, pine flatwoods, prescribed burning

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Abstract

Limited understanding of the ecological needs of the federally endangered Florida bonneted bat (*Eumops floridanus*, hereafter EUFL) restricts our ability to develop strategies to maintain or enhance habitat for the species. Although fire shapes vegetation communities across the species entire geographic range, the effects of prescribed burns on these bats are completely unknown. Fire may help or hinder EUFL, through direct or indirect pathways such as changes to roost tree abundance, understory or midstory vegetation, and insect prey availability. We investigated long- and short-term response of EUFL to prescribed fires to develop much-needed recommendations regarding fire management for this newly listed species endemic to south Florida.

We used an observational approach to examine the effects of historical fire frequency and seasonality on bat activity. We took a stratified random selection of 149 sites spread across a landscape gradient of historical fire frequencies (calculated over the previous 18 years), in four study areas managed regularly with prescribed fire. We repeatedly surveyed bat activity acoustically at each of these sites during two 6-month periods in 2015 and 2016. Variation in bat activity was best explained by both fire frequency and season: EUFL activity decreased with early wet season burn interval and increased with dry season burn interval. Bat activity and foraging activity were highest in sites burned at 3-5 year intervals during the historic fire season (the early wet season). We conclude that prescribed fires during the early wet season at this moderate frequency lead to favorable effects on habitat for EUFL, and recommend further studies to determine which mechanisms are responsible for these patterns.

We conducted a pre-post, treatment-control experiment in four prescribed burns at two study areas during two seasons (dry, wet) in 2015 and 2016 to assess short-term responses of EUFL to fire. We surveyed bat activity acoustically for 12 nights pre-burn and 24 nights post-burn, at three locations within burns (treatment sites) and three sites in nearby un-burned locations (control sites). We used generalized linear mixed-effects models to test the effect of fire treatments on bat activity, and assessed how bat activity changed temporally post-burn. Bat activity significantly increased immediately post-burn in treatment sites relative to control sites, with more pronounced effects during burns in the dry season than the wet season. While a negative trend in the burn effect size on bat activity was evident over time post-burn, the overall effect was positive. We conclude that burns have short-term positive effects for EUFL and recommend additional research to determine mechanisms underlying these trends.

We originally proposed an assessment of roost switching behavior of EUFL as a short-term response to prescribed burns, but were not able to accomplish this. In pursuit of this intent, we tested the use of an acoustic lure to draw EUFL to mist nets so we could attach radio transmitters to bats. Using this lure, and augmenting these efforts with captures at bat houses, we attached radio transmitters to 24 EUFL during the study period. This led to the discovery of eight natural roost structures. Although we were unable to directly tie the information obtained on these roosts to prescribed fire events as initially planned, these roost discoveries are noteworthy because they substantially increase understanding of the species habitat needs. Seven of the eight roosts were located in fire-maintained vegetation communities, and when these areas are burned can be protected and monitored for changes to roost use by biologists and land managers.

We conclude that EUFL appear to be fire-adapted, and benefit from prescribed burn regimes that closely mimic historical fire patterns. We encourage consideration of both fire frequency and seasonality when managing uplands with fire within the geographic range of this species.

Objectives

Our first objective was to examine relationships between EUFL activity and burn history with the goal of informing management decisions and policy guidelines. We used an observational approach to examine EUFL activity across a vast landscape gradient of fire frequencies. Because EUFL have evolved in a region adapted to fire, we predicted that bats would select habitat based on both the frequency and season of fires, favoring historical fire frequency and seasonality.

Our second objective was to examine short-term response of EUFL to fire. We conducted a pre-post, treatment-control experiment to determine the immediate effect of prescribed burns on bat activity, and to evaluate how this effect changes over time following prescribed burns. We predicted that bat activity would show a stronger immediate positive response to burns applied during the wet season than during the dry season, since wet season burns correspond to the growing season and tend to promote herbaceous vegetation (favored by many invertebrate prey) whereas dry season burns correspond to the dormant season which tend to promote woody vegetation. We also predicted there would be an initial decrease in bat activity post-burn, followed by an increase as insects and bats recolonized the burned areas.

Background

Little is known about the distribution, habitat requirements, and natural history of EUFL. This species is believed to have the most limited geographic distribution of any species of bat in the U.S. (Belwood 1992), although even the exact size of the species range is uncertain (FWC 2011, USFWS 2013).

The species was listed as endangered in October 2013, based on the determination that EUFL occur in limited numbers in a restricted range and face considerable and immediate threats (USFWS 2013). The threats facing the species include small population size, limited geographic range, low fecundity, relative isolation among populations, and vulnerability to extreme weather events (USFWS 2013). Given the rapid pace of urban development in south Florida and the frequency of extreme weather events in this region, the severity of threats facing the species are not only considered high at present, but likely to increase in the future. There is an urgent need for improved understanding of response of the species to common land management activities, as well as improved knowledge of roost site and foraging area selection. This information will enable the crafting of recommendations for managers of conservation lands so they can minimize and mitigate adverse effects of their activities on this newly listed species.

Fire has played a critical role in shaping the vegetative communities found within the range of EUFL. Florida has the highest lighting strike frequency in the country (Christian et al. 2003), and historically both lightning and human-ignited fires were common in the southern part of the state (Abrahamson and Hartnett 1990). Both the pine and prairie communities of south Florida contain many fire-dependent plant species, and even the wet swamp communities of south Florida burn frequently (Abrahamson and Hartnett 1990). However, the frequency of fire in the state has declined drastically over the last century. In 1926 over 75% of the pinelands in Florida burned annually, whereas in 2003 this was reduced to less than 1% (Slapcinsky et al. 2010). This radical change in the natural disturbance regime creates changes in forest composition and structure (Heyward 1939, Brown and Smith 2000) and has led to declines in many of Florida's rare and endemic species (Van Lear et al. 2005, Slapcinsky et al. 2010). The current scarcity of EUFL may be partially due to changes in the vegetative or insect communities these bats depend on while foraging and roosting; no formal investigations have been made of the response of these bats to different fire regimes to verify whether this may be the case.

Recognition of the importance of fire has led to a marked increase in the use of prescribed burning in Florida during the last few decades (Fowler and Konopik 2007). Controlled burning provides benefits to wildlife by promoting forest regeneration, enhancing nutrient cycling, maintaining fire-dependent plant species, and reducing the risk of catastrophic wildfire through reductions in midstory vegetation and accumulated dead fuels. Fire in forest ecosystems is expected to provide benefits to cavity and crevice-roosting bats through potential creation of new snags that function as roost trees, increases in abundance and diversity of insect prey through promotion of greater herbaceous plant abundance and diversity at the ground level, and opening of foraging space beneath the canopy (Boyles and Aubrey 2006, Knapp et al. 2009, Armitage and Ober 2012). On the other hand, fire may reduce roost abundance by consuming or toppling existing roost structures (Morrison and Raphael 1993). Historically, EUFL are known to have roosted in both natural and anthropogenic structures, including cavities in pines, palms, rock outcrops, houses, and bat houses (Belwood 1981, Timm and Genoways 2004, USFWS 2013). Although little is known of the species roost preferences (only 1 natural roost had been identified during the period extending from 1979 to 2014), the species tree-roosting habits and limited geographic distribution in the peninsula of Florida undoubtedly place it at risk of considerable roost loss from both natural and anthropogenic impacts. Given the widespread use of prescribed burning as a management tool in south Florida, better understanding is badly needed regarding the effects of fire on these bats. Also, more information is needed regarding potential short-term risks associated with prescribed burning such as relocation of foraging activities in the wake of fires (Johnson et al. 2009, 2010). This may cause stress among females during pregnancy, cause mortality of flightless pups during lactation, and increase risk of predation by necessitating immediate and unanticipated changes in roosts and foraging areas any time of year.

The large expanse of publicly owned lands in south Florida and the complete lack of understanding of how routine land management practices may cause additional unintended creation or losses of potential roosts and relocation of foraging activities leaves the species vulnerable even on conservation lands. The fact that a large percentage of the acreage in the twelve counties where the species has been detected during the past decade are publically owned (i.e., Broward, Charlotte, Collier, Desoto, Glades, Hendry, Highlands, Lee, Miami-Dade, Monroe, Okeechobee, and Polk Counties) places a great deal of responsibility for the species' recovery on land managers in the region. This also provides substantial opportunities for managers to promote the species by maintaining and enhancing habitat over a large proportion of the species range, which will be possible only with increased knowledge of the species habitat requirements.

Materials and Methods

Objective 1: Examine relationships between Florida bonneted bat habitat use and burn history

We surveyed four study areas managed by state and federal agencies with extensive upland pine and dry prairie communities within the core range of EUFL (USFWS 2013). These areas were selected because each has experienced a range of fire treatments during the past two decades. They were Big Cypress National Preserve (BICY; 720,000 acres in Collier, Miami-Dade, and Monroe Counties), Fakahatchee Strand Preserve State Park (FSPSP; 74,000 acres in Collier County), Florida Panther National Wildlife Refuge (FPNWR; 26,400 acres in Collier County), and Babcock-Webb Wildlife Management Area (BWWMA; 66,000 acres in Charlotte County) (Figure 1). These areas contain a mix of slash pine (*Pinus elliottii*) flatwoods, cypress (*Taxodium ascendens* and *T. distichum)* communities, freshwater prairies, ponds, and hardwood hammocks. We focused on the two common fire-maintained vegetation communities: upland pine flatwoods (mesic, hydric), and prairies (wet, dry, marl). Presumed historical fire return intervals for these vegetation communities are 1-2 years for dry prairie, 2-3 years for wet prairie, 2-4 years for pine flatwoods, and 2-10 years for marl prairie (Florida Natural Areas Inventory 2010).

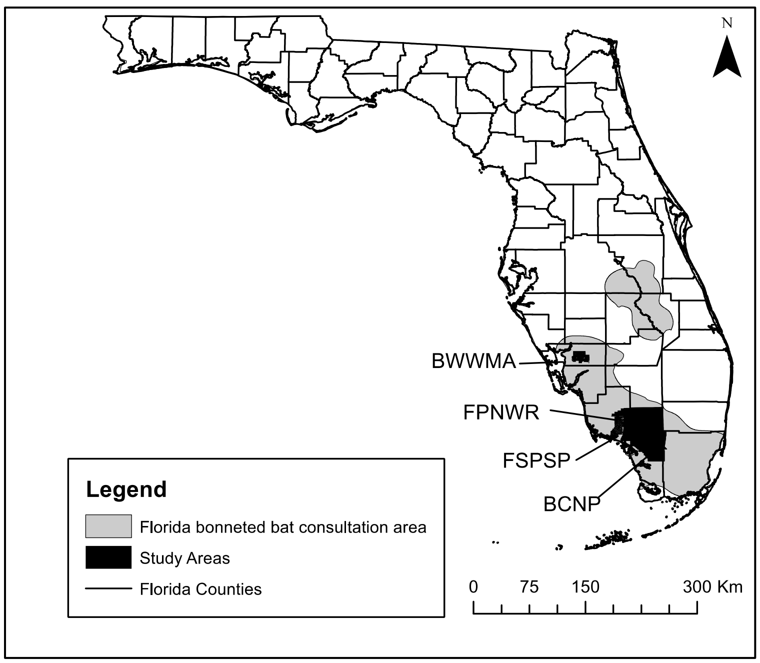

Figure 1. Four study areas (in black) where we conducted acoustic surveys in 2015 and 2016 in Florida to evaluate effects of prescribed burning on activity of EUFL. Gray shading depicts the consultation area for EUFL as defined by the United States Fish and Wildlife Service at the time the project began (USFWS 2013).

The study region experiences two primary seasons characterized by precipitation and temperature: dry/cool and wet/warm seasons (Duever et al. 1994, Slocum et al. 2010). A third season, the historical fire season (referred to as the 'early wet season'), occurs at the transition of the dry and wet seasons when conditions are most favorable for the spread of fire (Platt et al. 2015). Frequent lightning strikes, high temperatures, and an abundance of low moisture fuels combined with vegetation in the active growth phase create a period when the ecological benefits of burning may be maximized (Knapp et al. 2009). Fires during this season are most effective at controlling understory woody vegetation and regenerating herbaceous vegetation (Robbins and Myers 1992, Waldrop et al. 1992, Streng et al. 1993), whereas fires during the dry/cool season tend to favor woody plant regeneration due to the ability of such plants to mobilize carbohydrates stored in roots while dormant (Robertson and Hmielowski 2014).

We used acoustic surveys of bat activity coupled with fire history data reported by managers to assess the relationship between fire regime and bat activity. We used a stratified random approach to select sites, using regional land cover maps (Florida Cooperative Land Cover Map v3.1) and 18 years of burn history data obtained from land managers to calculate historical fire frequencies. After categorizing each site as belonging to one of three fire frequency categories (high frequency = 1-3 year fire return interval, moderate frequency = 3-5 year interval, and low frequency = greater than 5 year interval) (Darracq et al. 2016). We surveyed 149 sites (77 in 2015; 72 in 2016), with ~18 sites in each study area each year (9 in pine flatwoods and 9 in prairies). Sites were randomly selected such that they occurred ≥100m from the edge of each fire management unit or vegetation community, and ≥300m from other sites. To account for temporal variation in bat activity, we surveyed each site for 2 consecutive nights 4 times between January and July, with each survey separated by ≥21 nights, yielding 8 detector survey nights per site.

Bat activity at each location was recorded using stationary acoustic detectors (Song Meter SM3BAT with SM3-U1 ultrasonic microphones, Wildlife Acoustics, Inc., MA). Each microphone was stationed 3 m above ground level and orientated to maximize detection of high flying bats. Recorders were programmed to record from 30 mins before sunset to 30 mins after sunrise, and to record files of 15 sec duration.

We used Kaleidoscope Pro 3.14B (Wildlife Acoustics, Inc.) to differentiate noise from sounds produced by bats, for initial species classification, and to manually review the spectrograms of acoustic files. We used the ‘Bats of Florida Classifier’ (beta version) to identify EUFL calls. We required that each ‘bat file’ contain ≥2 bat calls, and used the number of bat files per night as an index of bat activity (Britzke et al. 1999, Tibbels and Kurta 2003, Davidai et al. 2015). To reduce species identification error and erroneous classification of lower quality bat calls, we manually reviewed 100% of the files classified by Kaleidoscope Pro as EUFL, NoID, or Noise. We also reviewed all ambiguous files for which the software provided multiple species identifications that included EUFL. We considered those files that contained calls with a characteristic frequency (fc) of 10-18 kHz and maximum frequency (fmax) of 16-22 kHz as EUFL (Bailey et al. 2017). We were conservative in our classifications, excluding calls that overlapped with the range of fc for Brazilian free-tailed bats, *Tadarida brasiliensis*, (18-33 kHz) (Szewczak 2011), or that may have been social calls from non-target bat species, acoustic echoes, insects, birds, or electronic noise. We further examined all files identified EUFL to differentiate those containing feeding buzzes, reflective of foraging activity (Fenton 1970, Coleman and Barclay 2013). To reduce subjectivity, two researchers experienced in bat call identification independently confirmed all manually validated files.

We determined the number of fires that occurred at each survey site during the previous 18 years using ArcGIS and the statistical software R (v. 3.3.3) with R studio (v. 0.98.1102). We calculated the mean number of years between burns (*BurnInterval*) and number of years since the last burn (*TimeSinceLastBurn*). We assigned a value of 20 years for both variables if no burns occurred at a site during the 18-year period. We assessed seasonality using operational definitions of three seasons from previous research in the region: Wet Season (June 1 – Nov. 1), Dry season (Nov. 2 – March 31), and Early wet season (April 1 – June 30) (Slocum et al. 2010, Platt et al. 2015). To address questions related to seasonality, we calculated *BurnInterval* and *TimeSinceLastBurn* for burns occurring during each season.

To evaluate relative habitat use by EUFL in relation to fire regimes, we quantified bat activity (number of bat call files) and foraging activity (number of bat feeding buzz files) detected per night at each site. We first tested for spatial autocorrelation among sites using a Mantel test (Mantel 1967), and finding no spatial autocorrelation ($Z = 0.021$, $P = 0.32$), we pooled all sites for further analyses.

We used generalized linear mixed-effects models (GLMMs) (function glmmadmb, R package glmmADMB; (Skaug et al. 2012), with "site" (detector location) as a random effect to account for multiple survey nights at each detector site. We first tested for an effect of survey year (2015, 2016) or vegetation community (pine flatwoods, prairies) on EUFL activity using a likelihood ratio test (function anova, R Stats package); as there was no effect of either variable (year: $\chi = 0.12$, $p = 0.729$; vegetation community: $\chi = 1.42$, $p = 0.233$), we pooled all survey data. We fit a null model and alternative models to a negative binomial distribution (zero inflated) with the following predictors: *BurnInterval*, *TimeSinceLastBurn* for all burns, and for burns conducted during each season (*WetBurnInterval*, *TimeSinceLastWetBurn*, *DryBurnInterval*, *TimeSinceLastDryBurn*, *EarlyWetBurnInterval*, *TimeSinceLastEarlyWetBurn*). We created a suite of models that included biologically relevant combinations of single variable, additive, interactive, and quadratic fixed effects. To avoid collinearity, we only included predictor variables in the same model that had correlation coefficients <0.5 (Booth et al. 1994, Zuur et al. 2009). We used Akaike's Information Criterion (AIC) and Akaike weights (ωi), to determine the relative support for each model (Burnham and Anderson 2002). All parameters were estimated using maximum likelihood (ML) and Laplace approximations to allow for model comparisons (Bolker et al. 2009, Pinheiro et al. 2012). To evaluate the effect of fire regime in a context relevant to existing prescribed burn management programs, we then conducted post-hoc analyses of the variables determined to be most important and divided our continuous burn interval variables into three commonly used burn interval categories (1-3 years, 3-5 years, and >5 years; (Darracq et al. 2016)). We constructed a GLMM with burn interval category as the predictor and bat activity as the response. To assess if bats were foraging differentially among sites in different burn interval categories, we also constructed a GLMM with foraging activity as the response. We tested the effect of categorical predictors by using a likelihood ratio test to compare two nested models (LRT, function anova, R Stats package; significance threshold $\alpha = 0.05$). We conducted Tukey pair-wise comparisons between levels of significant factors using the function glht (R package Multcomp) to adjust significance values for multiple comparisons.

Objective 2a: Examine short-term response of EUFL to fire: bat activity

Data were collected at two study sites within the core range of EUFL (USFWS 2013). The first was BWWMA and the second was FPNWR, both previously described under objective 1. This research occurred in the fire-maintained mesic and hydric pine flatwoods (ArcMap v10.2.2; Florida Cooperative Land Cover Map v3.1).

We coordinated with local fire managers to schedule our investigations to coincide with their plans for one prescribed burn during the wet season and one prescribed burn during the dry season at each study site. The dates of the prescribed fires in FPNWR were 21 February 2016 (dry) and 15 July 2016 (wet), and in BWWMA were 22 March 2016 (dry) and 25 June 2015 (wet) (Figure 2).

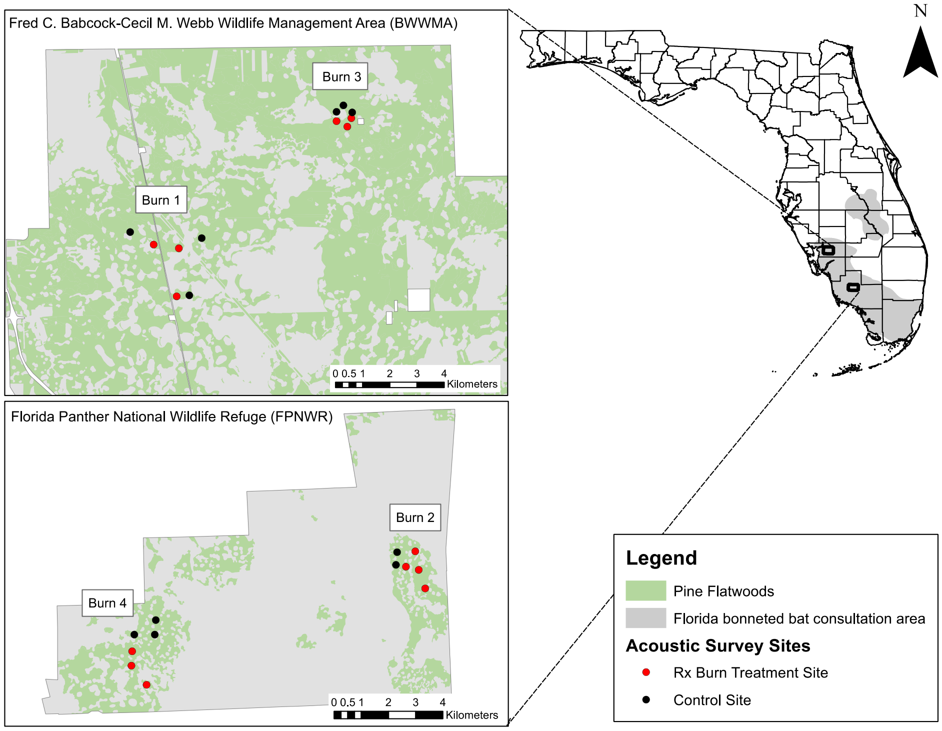

Figure 2. Map on right shows the location of the two study areas (black squares) where we conducted an experiment in 2015-2016 to test the effects of prescribed burns on activity of EUFL. Gray shading depicts the consultation area for EUFL as defined by the USFWS (USFWS 2013). Each of four prescribed fire events had six acoustic survey sites, with 3 that typically received burn treatments and 3 that typically served as control sites and were not burned.

At each burn, bat activity was surveyed acoustically at 3 randomly locations within the burn area (treatment) and 3 randomly selected locations immediately outside the burn area in physically similar locations (control). Each survey location was ≥100m from the edge of each fire management unit or vegetation community (pine flatwoods), and ≥300m from all other survey locations. We attempted to survey for 4 weeks prior to each burn and 4 weeks following each burn. Bat activity at each location was recorded and EUFL calls identified using the same methods described under objective 1.

We used generalized linear mixed-effects models within an information-theoretic model selection approach to compare bat activity before and after prescribed burns in treatment sites relative to control sites, and to compare temporal patterns in bat activity post-burn in treatment sites relative to control sites. All graphical and statistical analyses were conducted in the statistical software language R (v. 3.1.2) in conjunction with R studio (v. 0.98.1102). After standardizing the number of post-burn survey nights to the number of pre-burn survey nights by selecting the closest 12 complete survey nights before and after each burn, we pooled the four burns together and used "site" (detector location) nested within "burn" (individual burn) as a random effect to account for spatial and seasonal variation associated with each burn and multiple survey nights at each detector. We fitted a null and alternative models to a negative binomial distribution (zero inflated data) with categorical predictors to reflect timing (differentiating pre-burn from post-burn), and treatment (differentiating burn from control). We used AIC and Akaike weights (ωi) to select model(s) receiving the most support (Burnham & Anderson 2002). We tested the effect of predictors by comparing two nested models using a likelihood ratio test (function anova, R Stats package) and conducted post-hoc Tukey pair-wise comparisons between levels of factors using the function glht (R package Multcomp), to adjust significance values for multiple comparisons. Lastly, to investigate trends in bat activity post-burn in treatment sites relative to control sites, we used data from surveys 4-28 nights post-burn and tested the association between burn effect size (treatment – control) and the number of nights post-burn by constructing a linear mixed-effects model (Gaussian distribution), with "burn" as the random effect. We tested the significance of the predictor (nights post-burn) using a likelihood ratio test.

Objective 2b: Examine short-term response of EUFL to fire: bat roosts

We used two techniques to capture EUFL to attach radio transmitters and track their roost use. First, we captured bats as they emerged from bat houses at BWWMA. We set up stacked triple-high mist nets (Avinet, Inc., Dryden, New York) surrounding bat houses, opening the nets at sunset and leaving them open for a maximum of 3 h. The nets were monitored continuously and each entangled bat was carefully removed from the net and placed temporarily in a cotton bag until processed. Second, we used a BatLure™ (Apodemus Field Equipment, Mheer, Netherlands) to broadcast pre-recorded EUFL calls with an ultrasonic speaker, a programmable timer, and a secure digital card loaded with pre-recorded call files (Braun de Torrez et al. 2017). These devices were programmed to play EUFL social call files we previously recorded at EUFL roosts at BWWMA or FPNWR. This lure attracted bats to lower altitudes, enabling us to capture free-flying individuals that we could then track to previously unknown roost structures.

Each radio transmitter (model LB-2, Holohil Systems Ltd.) was sewed onto a break-away collar. Radios were attached to 16 individuals at BWWMA (nine in 2015 and 7 in 2016), 6 individuals in FPNWR in 2016, and 2 individuals in FSPSP in 2016. All individuals were either adult males or non-reproductive, adult females. Each individual was released at the site of capture directly following transmitter attachment, and tracked for approximately two weeks.

Results and Discussion

Objective 1: Examine relationships between Florida bonneted bat habitat use and burn history

We recorded acoustic data from 149 sites across 1,192 detector nights between January and July in 2015 and 2016. After eliminating acoustic files associated with nights when data were not recorded throughout the entire night due to equipment malfunction (10 detector nights), we identified 238,841 files that contained bat call sequences. Of these, we identified 4,783 files as containing EUFL calls (2.0% of total bat files). We detected EUFL at 147 of our 149 sites, and on 65.4% of detector nights. Mean EUFL activity was 4.0 ± 0.2 files per night and mean foraging activity per night was 0.07 ± 0.01 feeding buzzes per night.

The best model for explaining EUFL activity included two fixed effects: *EarlyWetBurnInterval* and *DryBurnInterval*. In accordance with our predictions, *EarlyWetBurnInterval* was negatively associated with bat activity (-0.03 ± 0.01, $p = 0.015$; Figure 3A). In contrast, *DryBurnInterval* exhibited a quadratic relationship in which it was positively associated from 0 to 10 years (9.03 ± 2.97, $p = 0.002$) and then negatively associated after 10 years (-6.78 ± 2.91, $p = 0.020$; Figure 3B). In contrast to what we predicted, *BurnInterval* and *TimeSinceLastBurn* were not included in the top models and were not significant predictors when tested in single variable models (*BurnInterval*: 0.01 ± 0.02; $p = 0.49$; *TimeSinceLastBurn*: -0.04 ± 0.03, $p = 0.200$).

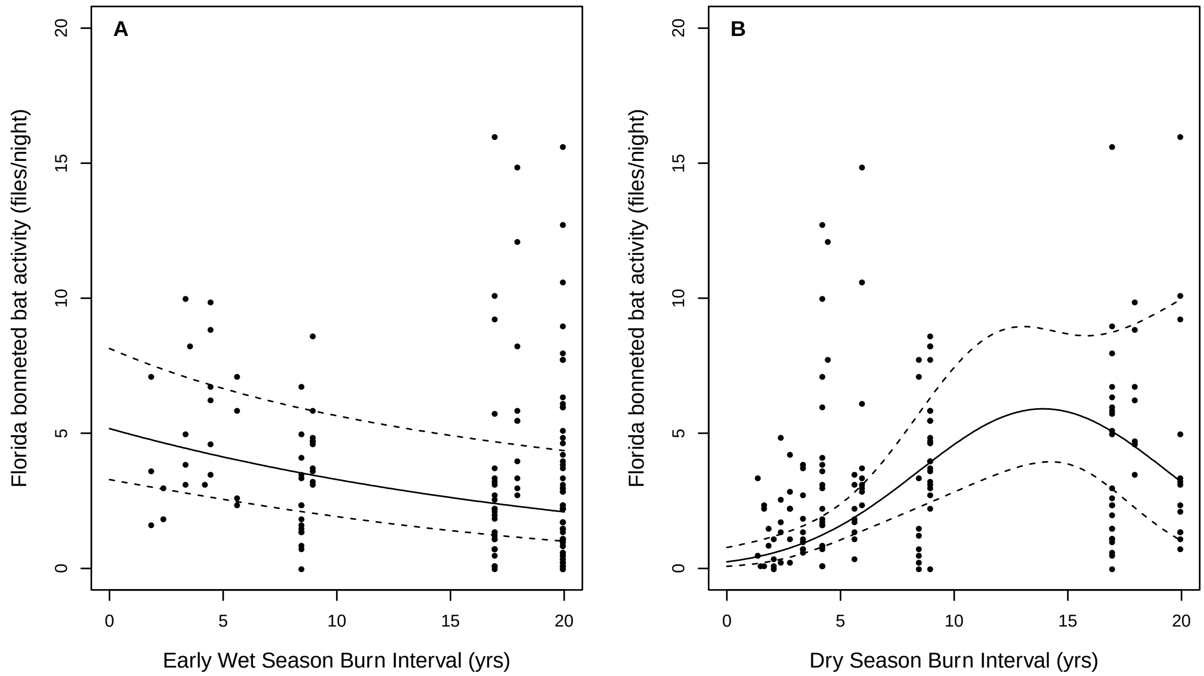

Figure 3. Association between activity of EUFL (acoustic files per night) and burn interval (mean number of years between burns) conducted within (A) the early wet season (April 1 – June 30) and (B) the dry season (November 2 – March 31). Lines represent fitted values (solid) and 95%CIs (dashed) generated from our best fit model. Points represent mean bat activity recorded at each site during 8 nights.

A post-hoc analyses of the predictors in our best model showed that bat activity differed among the three burn interval categories for each of the two seasons (*EarlyWetBurnInterval* LRT: $\chi2 = 10.98$, $p = 0.004$; *DryBurnInterval* LRT: $\chi2 = 13.78$, $p = 0.001$). After accounting for variation associated with *DryBurnInterval*, bat activity was higher in sites burned during the early wet season at a moderate frequency (>3-5 years) than at a low frequency (>5 years; Est. = 1.06 ± 0.30, $p = 0.001$), with no significant differences between the other pairwise category comparisons (Figure 4A). After accounting for *EarlyWetBurnInterval*, bat activity was higher in sites burned during the dry season at a low frequency than moderate (Est. = 0.53 ± 0.22; $p = 0.044$) and high frequencies (Est. = 0.80 ± 0.24, P = 0.002), with no difference between moderate and high frequencies (Est. = 0.27 ± 0.33, $p = 0.676$; Figure 4B). Foraging activity followed a similar pattern for burns conducted during the early wet season, though detections of feeding buzzes were low ($n = 80$): foraging activity was higher in sites burned during the early wet season at a moderate frequency than at a low frequency (Est. = 1.06 ± 0.46, $p = 0.048$), with no significant differences between the remaining pairwise comparisons. There were no significant pairwise differences in foraging activity among any burn interval categories for burns conducted during the dry season.

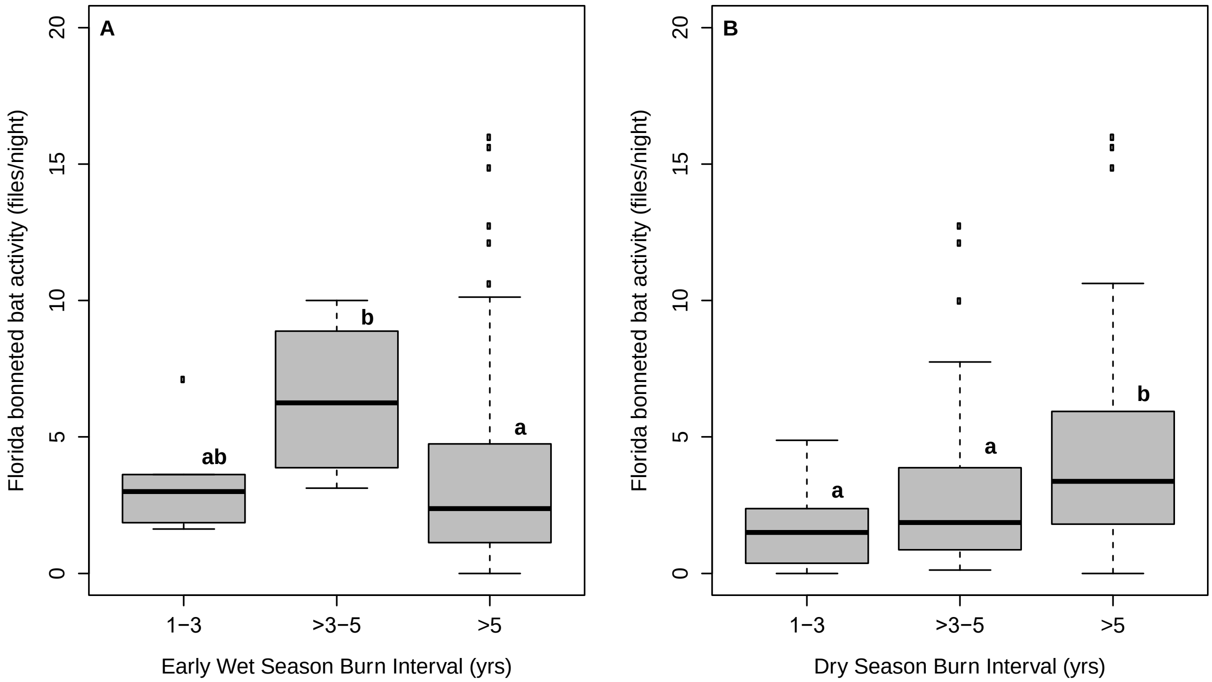

Figure 4. Comparison of activity of EUFL (acoustic files per night) and burn interval within (A) the early wet season (April 1 – June 30) and (B) the dry season (November 2 – March 31). Letters indicate significant differences in bat activity among categories.

Both the frequency and season of fires occurring in a site explained variation in activity of EUFL across the landscape. Bat activity and foraging activity were highest in sites burned at a moderate frequency (3-5 year burn interval) during the early wet season, which is consistent with the historical fire regime for this fire-adapted region. In contrast, burning frequently (short burn intervals) during the dry season appeared to have negative effects on EUFL activity. This lends support to the idea that fire-adapted species respond most favorably to burns conducted during the historical fire season (Knapp et al. 2009; Fill et al. 2012, Platt et al. 2015). Such patterns have been documented within the Southeastern US for a variety of flora and fauna (Sparks et al. 1998; Knapp et al. 2009).

Our finding that both bat activity and foraging activity were highest at a moderate burn interval of 3-5 years aligns with a recent meta-analysis, which found that vertebrate species richness in long-leaf pine ecosystems was maximized under these conditions (Darracq et al. 2016). This fire return interval may encourage use by a diversity of species due to the habitat heterogeneity and consequent niche space associated with this burn interval, which is characterized by a hardwood midstory (Hiers et al. 2014, Lashley et al. 2014). Foraging activity by EUFL suggests that insect availability may also be high under these conditions. In contrast, shorter burn intervals may create a more homogenous landscape with fewer resources, due to a sparse understory, less structure, and fewer fire-tolerant species (Ware et al. 1993, Engstrom et al. 2001, Lashley et al. 2014), whereas longer burn intervals may lead to reduced flight space for large bats (Armitage and Ober 2012) due to a dense hardwood midstory (Varner et al. 2005). The frequency and intensity of burns may also represent a tradeoff between creation of new tree cavities and destruction of existing snags (Perry 2012). Considering seasonality, sites that are burned at moderate frequencies within the early wet season may provide optimal conditions of flight space, roost availability and insect composition for bats.

Objective 2a: Examine short-term response of EUFL to fire: bat activity

We recorded 864 detector nights from 24 detector locations across the four prescribed burns in 2015 and 2016. After eliminating files from nights when data were not recorded throughout the entire night and removing files from one site entirely due to failed post-burn recording (a control site in burn 4), our final dataset included 129,561 acoustic files. Of the 79,305 files that contained bat call sequences, we identified 3,305 files as containing EUFL calls (4.2% of total bat files). We detected EUFL at every detector site and on 72.4% of detector nights. Mean EUFL activity was 4.26 ± 0.27 files per night and foraging activity (feeding buzzes) was 0.036 ± 0.007 files per night. Activity of EUFL varied by individual burn and treatment (Table 1).

Table 1. Activity of EUFL (mean calls/night ±SE) before and after prescribed fires at treatment sites (areas that received prescribed fire treatments) and control sites (areas that did not receive prescribed fire treatments)

Burn	Study Area	Year	Season	Pre-Burn Control Activity	Post-Burn Control Activity	Pre-Burn Treatment Activity	Post-Burn Treatment Activity
1	BWWMA	2015	Wet	0.81 ± 0.18	1.18 ± 0.52	1.25 ± 0.34	2.49 ± 0.71
2	FPNWR	2016	Dry	2.83 ± 0.73	2.46 ± 0.50	3.20 ± 0.58	8.06 ± 1.02
3	BWWMA	2016	Dry	1.58 ± 0.30	3.28 ± 0.50	3.39 ± 1.05	12.28 ± 3.73
4	FPNWR	2016	Wet	4.42 ± 0.69	2.00 ± 0.40	2.81 ± 0.32	1.58 ± 0.38

The best model explaining variation in bat activity was the PrePost*Treatment model which included an interaction term. Using post-hoc pair-wise comparisons, we found that mean bat activity was significantly higher during the post-burn period compared to the pre-burn period in burn treatment sites (Pre-Burn vs. Post-Burn: 0.67 ± 0.25, z = 2.692, p = 0.028), but was not significantly different during these periods in control sites (Pre-Control vs. Post-Control: 0.11 ± 0.18, z = 0.912, p = 0.912) (Figure 5). Similarly, bat activity levels in burn treatment sites and control sites were not significantly different during the pre-burn period (Pre-Control vs. Pre-Burn: 0.14 ± 0.39, z = 0.361, p = 0.978), but were significantly higher in burn treatment sites than control sites following burns (Post-Control vs. Post-Burn: 0.71 ± 0.27, z = 2.609, p = 0.036). This pattern of an increase of bat activity in treatment sites relative to control sites post-burn was evident across three of the four burns (burns 1, 2, and 3; Table 1). In burn 4, activity in the burned site remained lower than the control after the burn, but the difference in activity between treatment and control was reduced.

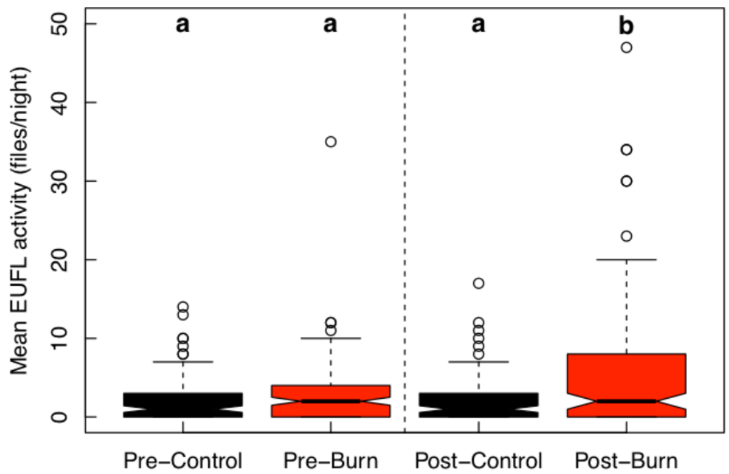

Figure 5. Mean EUFL activity prior to prescribed burns (pre) and after prescribed burns (post), in control sites (areas that did not receive prescribed fire treatments) and burn sites (areas that did receive prescribed fire treatments).

We also found that EUFL activity was variable among nights during the 4 - 28 nights following prescribed burns in treatment sites relative to control sites (Figure 6). The burn effect size decreased over time following fires, showing a weak negative association between the burn effect and number of nights post-burn treatment (-0.20 ± 0.10, t = 1.902; $\chi 2 = 3.587$, $p = 0.058$).

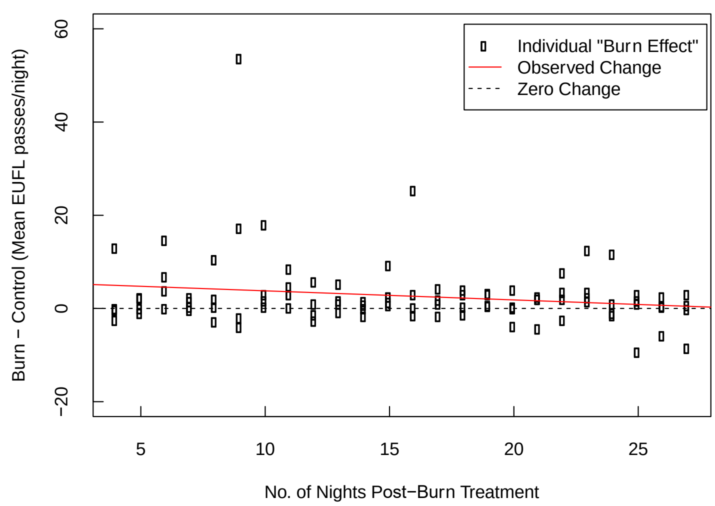

Figure 6. Temporal trend in the “burn effect”, which is the difference in mean EUFL activity (acoustic files/night), recorded in burn treatment sites relative to control sites after prescribed fire treatments, for four burn experiments combined. Positive values indicate higher bat activity in burn treatment sites relative to control sites on a given night. The red line depicts the relationship between the burn effect and nights post-burn, fitted with a linear mixed-effects model. The negative slope indicates a decrease in the effect of the prescribed fire treatment over time.

Our results suggest that EUFL were attracted to burned areas immediately following prescribed fires. These findings are not in line with previous assumptions that large-bodied bats, adapted to open or high-altitude flight, would be less affected by local changes to understory vegetation and insect prey following fire due to their flight high above the forest canopy (Buchalski et al. 2013). The apparent effects of fire on EUFL that we found support studies from bat assemblages in other regions which show that forest bat activity either increases or does not change in response to burns (Loeb and Waldrop 2008, Armitage and Ober 2012, Perry 2012, Buchalski et al. 2013). Our research adds additional evidence that bats are resilient to, and perhaps benefit from, fire.

Our study is unique in that we used an experimental approach. Previous research has used correlations or post-fire site comparisons over varying periods or time following burns. We are not aware of any pre-post, treatment-control experiments that documented changes in bat activity directly before and after a fire, or examined temporal patterns in the bats' immediate response as we have done.

The two burns that occurred during the dry season (spring) provoked a stronger response by EUFL than did the two burns conducted in the wet season (summer). Bats may be attracted to the concentrated insect at freshly burned sites during the cooler dry season when prey is limited. This is also likely an energetically demanding time, when EUFL are in the early stages of pregnancy (Ober et al. 2017). In contrast, temperatures and insect prey availability are higher within this region during the wet season (Amalin et al. 2009). Thus, bats may not be as prey limited during summer, and selection of prime foraging sites may be less critical. But with a sample size of only four prescribed burns, we are limited in our ability to conclude that the observed differences across burns were due to season rather than to some other uncontrolled factor associated with each burn.

Contrary to our predictions, we did not observe an initial decrease in EUFL activity following prescribed burns. Rather we found an increase in bat activity immediately after the burns and subsequent decrease over time, suggesting that the initial positive effect of prescribed burns on EUFL is temporary. This could be due to the initial attraction by insects to fires as they burn and smolder, before subsiding over time. With no studies that examine temporal patterns of bat activity immediately after a fire, it is difficult to put our results in context. Because our access was restricted to burned sites during the first four days following each burn, we may have missed any initial reduction in bat (and insect) activity that occurred during the first three nights as a result of fire.

Objective 2b: Examine short-term response of EUFL to fire: bat roosts

Using radio-transmitters, we tracked several bats to natural roost structures (Table 2). At BWWMA, male bats led us to two live slash pine trees and to three slash pine snags, while a female led us to an abandoned, enlarged RCW cavity in a live slash pine tree. Capture efforts in FSPSP allowed us to follow male bats to a royal palm snag in FSPSP and to a slash pine snag we had identified as a roost in FPNWR several months prior, using acoustics (Braun de Torrez et al. 2016). Capture efforts at FPNWR allowed us to track two males that led us back to the royal palm snag in FSPSP.

Due to the difficulty of identifying a large enough sample size of roost structures during our study period, we were unable to tie our roost findings to prescribed fire events as we originally intended. However, the development of methods to effectively capture and track EUFL to new roost locations was a significant accomplishment in itself (Braun de Torrez et al. 2017). Previously, 465 mist-net nights (using triple-high mist-net sets) of effort yielded only one EUFL capture, despite nets being set in areas where the species had been documented to occur. Further, these eight roosts represent a substantial increase in understanding of the species habitat needs, given that only a single natural roost was known when we began our research in 2014 (Angell and Thompson 2015). Seven of the eight roosts (all slash pine trees and snags) were located in fire-maintained vegetation communities, suggesting that fires are relevant to the maintenance of the structures these bats use as roosts. Land managers can now protect and monitor these roost structures to evaluate changes in roost use in response to events such as wildfires, prescribed burns, or other management actions. Our ability to capture and track free-flying EUFL expands future research opportunities that can improve understanding of roosting and foraging ecology, and to direct conservation and management actions across the species' range.

Table 2. Location and type of trees and snags identified as natural roost structures for EUFL via radio-telemetry during 2015 and 2016.

Location of Roost	Type of Roost Structures Discovered
BWWMA	3 live slash pine trees and 3 slash pine snags
FPNWR	1 slash pine snag
FSPSP	1 royal palm snag

Conclusions and Implications for Management, Policy, and Future Research

Restoring fire to fire-dependent forests may contribute to enhanced foraging habitat for critically endangered EUFL. Our study provides land managers with the first evidence that EUFL may benefit from fire, and emphasize the importance of considering seasonality in addition to fire frequency when evaluating the effects of fire on biodiversity.

We suggest that EUFL are fire-adapted and benefit from prescribed burns that closely mimic natural historical fire regimes. We recommend that burns are conducted at the time of year when natural fires historically occurred (during the early wet season), and are conducted on a 3-5 year interval to provide the greatest benefit to this species. If burns can only be conducted during the dry season, they should be conducted less frequently (>5 year interval).

We encourage future research on the mechanisms driving responses of EUFL and other bat species to fire regimes.

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Appendix A: Contact Information for Key Project Personnel

PI - Dr. Holly K. Ober
Associate Professor
Department of Wildlife Ecology and Conservation
University of Florida
Holly.ober@ufl.edu
850-875-7150

Co-PI - Dr. Robert A. McCleery
Associate Professor
Department of Wildlife Ecology and Conservation
University of Florida
Ramccleery@ufl.edu
352-846-0566

Project Lead - Dr. Elizabeth C. Braun de Torrez
Postdoctoral Researcher
Department of Wildlife Ecology and Conservation
University of Florida
ecbraun@ufl.edu or Elizabeth.Braun@myFWC.com
617-895-7937

Appendix B: List of Completed or Planned Scientific, Technical Publications, and Science Delivery Products

Journal Articles

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. *In prep*. Recreating historical fire regimes increase activity and foraging of an endangered bat. To be submitted to Conservation Biology. (*Expect to submit in March 2018*).

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. *In review*. Activity of an endangered bat increases immediately following prescribed fire. Submitted to Journal of Wildlife Management (Submitted 10 October 2017).

Braun de Torrez, E.C., S. Samoray, M. Gumbert, M.A. Wallrichs, K. Silas, H.K. Ober, and R.A. McCleery. 2017. Acoustic lure allows for capture of a high-flying, endangered bat. Wildlife Society Bulletin 41: 322-328.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. 2016. Use of a multi-tactic approach to locate an endangered Florida bonneted bat roost. Southeastern Naturalist 16: 235-242.

Conference Presentations

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. *Anticipated.* Fire-adapted? Recreating historical fire regimes may benefit an endangered bat. North American Joint Bat Working Group Meeting. 27-29 March 2018, Roanoke, VA.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. Bats and fire: activity of endangered Florida bonneted bats (*Eumops floridanus*) increases immediately following prescribed burns. 7th International Fire Ecology and Management Congress. 28-30 November 2017, Orlando, FL.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. Bats and fire: Endangered Florida bonneted bats respond positively to prescribed burns. 47th Annual Symposium for North American Society for Bat Research. 18-21 October 2017, Knoxville, TN.

Braun de Torrez, E.C., S.T. Samoray, M.W. Gumbert, H.K. Ober, and R.A. McCleery. Acoustic lure allows for capture of a high-flying endangered bat: the Florida bonneted bat. 22nd Annual Meeting of the Southeastern Bat Diversity Network. 16-17 February 2017, Asheville, NC.

Workshop Presentations

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. Species-specific monitoring: Endangered Florida bonneted bats (*Eumops floridanus*). National Park Service Webinar on Acoustic Data Management for Bats. 12-14 Dec 2017.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. Current research on Florida bonneted bats, *Eumops floridanus*: acoustic surveys, social structure, roost location and effects of fire. Florida Bonneted Bat Working Group Meeting. 24-25 May 2016, Avon Park, FL.

Braun de Torrez, E.C. Overview of current research on Florida bonneted bats (*Eumops floridanus*) using acoustics and GPS tracking technology. Bat Conservation and Management (BCM) Acoustics Workshop. January 2016, Punta Gorda, FL.

Ober, H.K., E.C. Braun de Torrez, and R.A. McCleery. Managing with fire to promote the recently listed Florida Bonneted Bat, *Eumops floridanus*. Florida Bonneted Bat Working Group Meeting. 5-6 November 2014, Miami, FL.

Field Demonstrations

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. *Anticipated.* Managing with fire: Short and long-term effects of prescribed burns on Florida bonneted bats. Florida Bonneted Bat Working Group Meeting. 23-24 May 2018, Naples, FL.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. Pieces of the puzzle: effects of fire on the elusive Florida bonneted bat. Annual Big Cypress Research Symposium. 9 November 2017, Everglades City, FL.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. Research on the refuge: Florida bonneted bats and fire. Florida Panther National Wildlife Refuge Open House. 30 April 2016, Immokalee, FL.

Braun de Torrez, E.C., H.K. Ober, and R.A. McCleery. The truth about bats – Research on rare Florida bonneted bats. Naples Zoo Lecture Series. 21 January 2016, Naples, FL.

Fact Sheets

Ober, H.K., E. C. Braun de Torrez, and R. A. McCleery. *In prep*. Recommendations for managing with fire to promote the endangered Florida Bonneted Bat, *Eumops floridanus*.

Ober, H.K., E. C. Braun de Torrez, and R. A. McCleery. *In prep*. Recommendations for managing with fire to benefit Florida's bats.

Appendix C: Metadata

Data Types

To examine short-term and long-term response of EUFL to prescribed burns, we recorded EUFL activity via echolocation detectors. We stored all raw recorded echolocation calls as full spectrum WAV files, in folders labeled according to date and location recorded, with file names indicating time of night recorded.

For data used to address objective 1, we produced a workbook in Microsoft Excel indicating the date, time, and location of each EUFL echolocation call sequence recorded at each site, coupled with the following fire characteristics of each survey site (determined from historic records from the previous 18 years): time since last burn, time since last wet season burn, time since last dry season burn, time since last early wet season burn, burn interval (mean number of years between burns), wet season fire return interval, dry season fire return interval, early wet season fire return interval, vegetation community type. The file contains a metadata worksheet describing these data.

For data used to address objective 2a, we produced a workbook in Microsoft Excel indicating the date, time, and location each EUFL echolocation call sequence recorded at each site, coupled with the following characteristics of each survey: burn name, treatment type (burn or control), and number of days pre or post burn. The file contains a metadata worksheet describing these data.

For data used to address objective 2b, we produced a workbook in Microsoft Excel indicating the date each roost was discovered; the sex and identity of the individual bat that was radio-tracked to that structure; characteristics of the tree, such as tree species, status, height, tree DBH; characteristics of the exact location within the tree used by the bat, such as height and orientation of the roost entrance, width and height of the roost entrance; and the maximum number of bats counted at the roost.

Data Storage and Backup

Data are stored on a laptop hard drive, backed up on an external USB drive, and backed up on a University of Florida server associated with the Department of Wildlife Ecology and Conservation. We will deposit all three workbooks described above within the permanent archive within two years of the completion of the grant (i.e., by 28 February 2020). As of February 2018 we have one journal article in review and expect to submit a second within the next month. Fact Sheets will be produced once both journal articles are published, and these, plus the journal articles will be placed in the archive as well.

Data Access

Once the investigators have received full benefit from the data through journal publications we will make the data available to others. Raw data and metadata will be made public through the Institutional Repository of the University of Florida (http://ufdc.ufl.edu/ir).